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ORIGINAL RESEARCH

Insulin Injection Technique is Associated with Glycemic Variability in Patients with Type 2 Diabetes

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ABSTRACT

Introduction: Patients with type 2 diabetes (T2D) receiving premixed insulin often fail to achieve optimal glycemic control. The insulin injection technique (IT) itself may be one of the factors affecting glycemic variability (GV). The aim of this study was to assess the relationship between GV and IT in patients with T2D using premixed insulin.

Methods: This was a single center, cross-sectional, and self-controlled trial. Patients with T2D using premixed insulin were enrolled as inpatients. The 4-day study consisted of a 2-day patient insulin injection period (days 0 and 1) and a 2-day specialist nurse insulin injection period (days 2 and 3). Patient insulin IT were assessed on day 1 by two independent nurses using a skill-related scale consisting of 15 items, with a maximum score for each item of 2 and a total optimum score of 30. All patients underwent 96-h continuous glucose monitoring (CGM) during the 4-day study, and CGM data collected on days 1 and 3 were recorded and analyzed. The primary outcome was the relationship between the insulin IT score and the 24-h mean amplitude glycemic excursion (MAGE) during the patient injection period.

Results: A total of 52 inpatients with T2D who used premixed insulin were recruited and completed the study. The mean total insulin IT score of these patients was considerably lower than the optimum score (17.0 ± 4.4 vs. 30). Our CGM data showed that the MAGE was significantly higher during the patient injection period than during the nurse injection period ($P < 0.05$). Multiple linear stepwise regression analysis showed that the patient IT score was negatively correlated to the MAGE ($P < 0.05$). The patient IT score was also negatively correlated to glycated hemoglobin (HbA$_{1c}$; $P < 0.05$).

Conclusions: A poorer insulin IT may negatively affect GV and HbA$_{1c}$ control in patients with T2D using premixed insulin. Our data indicate that the insulin IT is important for short- and long-term glycemic control.

Clinical Trial Registration: ClinicalTrials.gov identification number: NCT03513055.

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INTRODUCTION

The incidence of diabetes in the world has increased dramatically during recent decades, with rapid economic development, aging populations, and lifestyle changes being cited as major contributory factors [1–3]. However, glycemic control remains a tough challenge worldwide [3]. One survey conducted in China showed that only 39.7% of patients with diabetes achieved adequate glycemic control (glycated hemoglobin [HbA$_{1c}$] < 7%) [3], while data from the USA showed that 50.1% of diabetic patients had an HbA$_{1c}$ level of < 7% [4]. Insulin is increasingly being used to manage diabetes in patients whose treatment with oral antihyperglycemic agents has failed. About 61.5% of Chinese patients with type 2 diabetes (T2D) are on insulin therapy [5]. Different from Western countries, premixed insulin is the common choice as the starter insulin preparation for Asian patients [2, 6, 7]. A retrospective database analysis of insulin therapy in Chinese patients with T2D revealed that premixed insulin was prescribed much more (77.3%) than basal (11.8%) and prandial (10.9%) insulin [8]. Various studies have found that an inappropriate insulin injection technique (IT) when using the insulin pen, such as incorrect rotation of the injection site and needle reuse, may affect the efficacy of insulin therapy, eventually leading to poor glucose control [9, 10]. Huang et al. further highlighted that the patients in their study on premixed insulin therapy failed to achieve optimal HbA$_{1c}$ control [11]. In a Turkish study, about 40% of patients using an insulin injector device had glycemic variability (GV) [12].

GV is associated with micro- and macrovascular complications [13]. For example, the level of circulating cytokines are higher under conditions of GV than when elevated glucose concentrations are stable [14]. Continuous glucose monitoring (CGM), with its capability of recording blood glucose fluctuations, represents a new tool for studying the influence of factors on GV in real life [15].

We have conducted a single-center, cross-sectional, self-controlled study to determine the relationship between GV and insulin IT in patients with T2D who used premixed insulin.

METHODS

This was a single-center, cross-sectional, self-controlled trial (ClinicalTrials.gov identification number: NCT03513055). The study protocol and patient consent forms were approved by the Institutional Ethical Committee of Nanjing First Hospital, Nanjing Medical University. All procedures followed were in accordance with the ethical standards of Nanjing First Hospital and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all participants included in the study.

Between May and July 2018, patients with T2D who used premixed insulin were enrolled as inpatients in the Department of Endocrinology, Nanjing First Hospital, Nanjing Medical University, China. All patients had been referred to the department by endocrinologists, and no patient came from a primary care center. The inclusion criteria were: (1) diagnosis of T2D according to World Health Organization criteria; (2) age between 18 and 80 years; (3) insulin delivered by insulin pen; (4) use of stable premixed insulin dose for at least 3 months; (5) patient willingness to undergo CGM; (6) patient self-injected insulin. Patients were excluded from the study if they met any one of the following conditions: (1) insulin was injected by a third party; (2) pregnancy, breastfeeding, or plans to become pregnant; (3) presence of acute metabolic diabetic complications, such as ketoacidosis or hyperosmolar state (coma); (4) presence of serious complications, such as cardiovascular disease, end stage renal disease, among others; (5) poor compliance and irregular eating and exercise; (6) presence of severe retinopathy or other eye problems that impair visual function; (6) cognitive dysfunction. All patients had received insulin injection

education when they were initially put on insulin therapy.

The study included a 2-day patient insulin injection period and a 2-day specialist nurse insulin injection period (Fig. 1). The demographic data for each patient were recorded, and fasting plasma serum was collected for the measurement of HbA_{1c} concentration on day 0. On days 0 and 1, patients injected themselves with their usual dose of premixed insulin, and the IT of each patient was assessed independently by two specialist nurses who used a scale composed of 15 skill-related items according to the New Insulin Delivery Recommendations and previous studies [16–19]. Each item was weighted with a score ranging from 0 to 2 points (Table 1), and the scores were averaged for further analysis. On days 2 and 3, the same type of insulin (unopened) was injected by nurses with the doses unchanged. During the study period, patients received premixed insulin twice daily, once before breakfast (0700 hours) and once before dinner (1700 hours). The abdomen was used for all injection sites. In this study, all patients received insulin with 6-mm pen needle.

CGM was performed for 96 h on all patients during the 4 days of the study using a Medtronic Inc. (Northridge, CA, USA) CGM system, as previously described [20, 21]. In addition, all patients were instructed to perform self-monitored blood glucose measurements, using an OneTouch Ultra Vue blood glucose monitor (LifeScan, Milpitas, CA, USA) at four time points per 24-h period: before breakfast, lunch, and dinner and before going to bed. After the CGM data were collected, the mean amplitude of glycemic excursion (MAGE) was calculated manually for each patient by measuring the arithmetic mean of the ascending and descending glycemic excursions between consecutive peaks and nadirs for the same 24-h period, and only absolute glycemic excursion values with a standard deviation (SD) of > 1 were considered, as previously described [20–22]. Other glucose indicators were also recorded, including the 24-h mean glucose concentration (MG), the 24-h SD of the MG (SD), the incremental area under the curve (AUC) of plasma glucose > 10.0 mmol/L and < 3.9 mmol/L, and the percentage of time in that the patient was in hypo-, normo- and hyperglycemia, respectively,. The primary outcome was the relationship between the insulin IT and MAGE. The secondary outcomes were the differences in glycemic profiles according to who injected the insulin (patient vs. nurse).

Statistical Analysis

The analyses were performed using the SPSS version 16.0 statistical package (IBM Corp., Armonk, NY, USA). All variables were tested for normal distribution of the data. Data were presented as the mean $\pm$ SD or as the median with the interquartile range (IQR; 25–75th percentile) according to whether the data were normally distributed or not. Square root transformation of the data was performed if the new transformed data achieved a normal distribution. The paired t test was used to compare two groups when the data were normally distributed; otherwise, Wilcoxon analysis was performed. Multiple linear stepwise regression
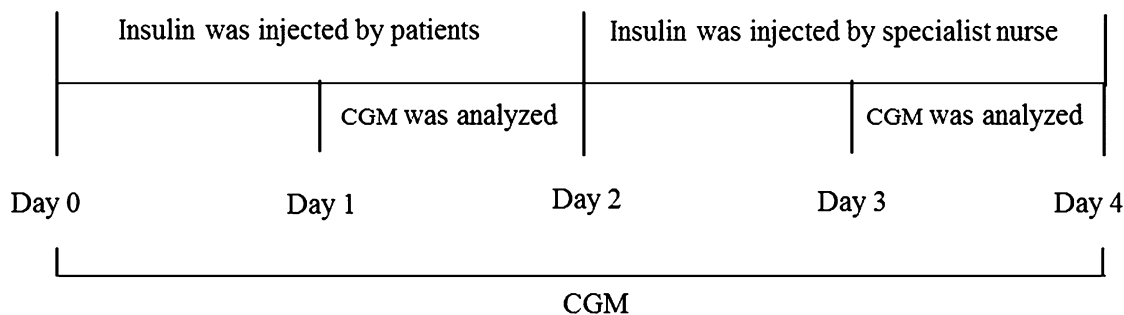
Fig. 1 Flow chart of the study. *CGM* Continuous glucose monitoring

Table 1 Items and scores of the premixed insulin injection technique

Items	Scores
Injection at indicated time	
Yes	2
No	0
Warming insulin up to room temperature before injection	
Yes	
> 30 min	2
< 30 min	1
No	0
Checking insulin (dosage and liquid) before injection	
Yes	2
Partly	1
No	0
Resuspension of insulin before injection	
Yes	
Correct	2
Incorrect	1
No	0
Attaching a pen needle	2
Correct	
Incorrect	0
Priming before injection	2
Yes	2
No	0
Inspection of injection sites	
Yes	2
No	0
Rotation of injection sites	2
Correct	2
Incorrect	0
Disinfecting the skin and injecting insulin after area of disinfection has dried	
Yes	
Correct	2
Incorrect	1

Table 1 continued

Items	Scores
No	0
Pinching the skin	
Yes	2
No	0
Needle entry angle	
Correct	2
Incorrect	0
The duration of time(s) the pen needle is under the skin	
> 10	2
5–10	1
< 5	0
Pulling needle out while holding dose knob	
Yes	2
No	0
Needle reuse (times)	2
1	2
2–5	1
> 5	0
Insulin (opened and unopened) storage	
Yes	2
Partly	1
No	0
Maximum score of all 15 items	30

Each item was weighted with a score ranging from 0 (minimum) to 2 (maximum) points

analysis was performed to identify parameters likely to correlation with MAGE or HbA_{1c}. Spearman's analysis was performed to identify correlation between each injection item and MAGE or HbA_{1c}. All comparisons were two-sided at the 5% significance level, and a P value of < 0.05 was considered to be statistically significant.

RESULTS

A total of 60 patients were initially recruited to this study, of whom two were excluded due to not meeting the self-injection criterion, one was excluded due to the development of sudden deafness which necessitated glucocorticoid therapy, and five were excluded due to missing

CGM data. Thus, the data of the 52 patients (27 males, 25 females) who completed the trial were analyzed at the endpoint. The mean age of these 52 patients was 63.8 ± 9.5 years, their mean HbA_{1c} level was $8.1 \pm 1.5\%$, and the mean body mass index (BMI) was 24.9 ± 3.2 kg/m^2. All patients had diabetes for a long period of time (159.9 ± 77.6 months), and all had used insulin for a mean of 60 (IQR 36–120) months. The mean daily insulin dose was 0.6 ± 0.2 U/kg/day.

The mean total patient IT score for the 15 skill-related items was lower than the optimum score (17.0 ± 4.4 vs. 30). Our CGM data showed that the glucose profiles, such as MAGE, SD, MG, and AUC > 10 mmol/L, and percentage of time in a hyperglycemic state were higher during the patient injection period than during the nurse injection period ($P < 0.05$, respectively). The percentage of time in the normoglycemic state in the patient injection period was significantly lower than that in the nurse injection period ($P < 0.05$). The AUC < 3.9 mmol/L and percentage of time in the hypoglycemic state were not significantly different between the two injection periods (Table 2).

To determine the relationship between insulin IT and the glycemic variability, we performed a multiple linear stepwise regression analysis, controlling for gender, age, BMI, HbA_{1c}, duration of insulin therapy, dosage of insulin used per day, duration of diabetes, and insulin IT score. Our data showed that the patient insulin IT score was significantly correlated with MAGE ($B = -0.29$, $P < 0.05$). Spearman's analysis showed that MAGE was correlated to the IT score for the items resuspension, pinching the skin, and needle reuse ($r = -0.48$, -0.35, and -0.47, respectively; all $P < 0.05$).

We also assessed the relationship between insulin IT and long-run glycemic control. Multiple linear stepwise regression analysis, controlled for gender, age, BMI, duration of insulin therapy, dosage of insulin used per day, duration of diabetes, and insulin IT score, showed that only patient insulin IT score was significantly correlated with HbA_{1c} ($B = -0.12$, $P < 0.05$). Spearman's analysis showed that HbA_{1c} was negatively correlated to the score for

the rotation of injection sites ($r = -0.33$, $P < 0.05$).

DISCUSSION

In this study, we found that only 27% of patients with T2D who received premixed insulin therapy achieved the desired HbA_{1c} target of $\leq 7\%$. The CGM data showed that the glucose profiles, such as MAGE, SD, MG, AUC > 10 mmol/L, and percentage of time in the hyperglycemic state, were significantly higher during the patient injection period than during the nurse injection period. The percentage of time in the normoglycemic state was significantly lower in the patient injection period than in the nurse injection period. Moreover, we found that both HbA_{1c} level and MAGE were negatively corrected to the insulin IT score, indicating that the insulin IT may play a role in short- and long-term glycemic control.

Diabetes patients worldwide commonly use an incorrect insulin IT, such as incorrect rotation of the injection site, incorrect needle reuse, incorrect (or no) resuspension procedure, among others [9, 10]. It is recommended that the vial or pen containing NPH (Neutral Protamine Hagedorn) insulin be tipped back and forth at least 20 times to resuspend the insulin, but few patients do this correctly, partly because the procedure is considered to be boring and unnecessary [18, 23]. However, resuspension of the insulin is important because an incorrect resuspension may result in the delivery of an inappropriate insulin dose [24–26], which may further alter the pharmacokinetics and pharmacodynamics of insulin, possibly leading to increases in daily GV [27]. It has been reported that resuspension training can decrease hypoglycemia [23] and the HbA_{1c} value [18]. Only five patients in our study resuspended the premixed insulin correctly. In this context, it should be noted that Spearman's analysis of our data showed that the resuspension score of our patients was negatively correlated to MAGE as determined by CGM, indicating that the correct resuspension procedure may decrease GV in patients with T2D who are receiving premixed insulin.

Table 2 The glucose profiles in the different injection periods

Parameters	Patient injection period	Nurse injection period	P value
MG (mmol/L)	9.5 ± 2.2	8.3 ± 1.9	0.00
SD (mmol/L)	2.2 ± 1.0	2.0 ± 0.9	0.03
MAGE (mmol/L)	5.4 (3.7, 7.9)	3.8 (2.8, 5.4)	0.00
AUC > 10 mmol/L (mmol/L/day)[a]	0.7 (0.1, 1.7)	0.1 (0.0, 1.0)	0.00
AUC < 3.9 mmol/L (mmol/L/day)[b]	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.13
Percentage of time in hyperglycemia (%)[c]	30.7 (9.8, 53.8)	13.7 (2.3, 37.1)	0.00
Percentage of time in normoglycemia (%)[d]	69.3 (46.2, 88.2)	86.1 (61.5, 95.1)	0.00
Percentage of time in hypoglycemia (%)[e]	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.10

Data are presented as mean ± standard deviation (SD) or as the median with the interquartile range (IQR; (25th, 75th percentile) in parenthesis

MG 24-h mean glucose concentration, *SD* standard deviation of MG, *MAGE* 24-h mean amplitude of glycemic excursion, *AUC* area under the curve

[a] AUC > 10 mmol/L is the incremental area under the curve of plasma glucose > 10.0 mmol/L

[b] AUC < 3.9 mmol/L is the incremental area under curve of plasma glucose < 3.9 mmol/L

[c] Percentage of time in hyperglycemia is the percentage of time the patient is is a hyperglycemic state (glucose level > 10 mmol/L)

[d] Percentage of time in normoglycemia is the percentage of time the patient is in a normoglycemic state (glucose level from 3.9 to 10 mmol/L)

[e] Percentage of time in hypoglycemia is the percentage of time the patient is in a hypoglycemic state (glucose level < 3.9 mmol/L)

Approximately half of diabetic patients worldwide use their needles more than once [9], a practice which is associated with unexplained hypoglycemia, hyperglycemia, increased GV, and slightly higher HbA_{1c} levels [10]. More recently, Ji and Lou confirmed a significant relationship between needle reuse and lipohypertrophy (LH) [17]. Authors of other studies have also reported that patients with LH had significantly higher GV (defined through self-monitoring of blood glucose) [17, 28, 29]. In our study, only 5.8% patients used their needles only once. Moreover, we identified that the needle reuse score was linked to MAGE.

Incorrect rotation of the injection site can result in LH, which may in turn result in an increase in the HbA_{1c} level in diabetic patients, in both Western and Chinese diabetic populations [9, 10, 30, 31]. In accordance with previous studies, our data showed that the score for the rotation of injection site was negatively correlated with the HbA_{1c} value.

In their worldwide study of injection practices, Frid et al. found that 63.7% of patients lifted a skinfold and that 75.0% of these did it correctly [9]. The great majority of patients in our study pinched the skin correctly. We found that the IT score for pinching the skin was negatively related to MAGE.

In this study, the total patient score for the insulin IT was calculated by summing the patient's score for each item. Analysis of the IT score revealed that both the HbA_{1c} level and MAGE were negatively correlated to the IT score.

The strength of our study is that we determined that correct insulin IT practices, such as resuspension of the insulin, pinching the skin, and rotation of the injection site, are important for achieving glycemic control in patients receiving premixed insulin therapy. These

results indicate that physicians and diabetes educators should pay more attention to developing and implementing programs aimed at educating diabetes patients on their insulin IT.

The present study has several limitations. First, it was a small-scale hospital-based observation study, and there was no follow-up study. Second, we did not observe an association between insulin IT and hypoglycemia; a follow-up study should be performed in the future to examine the relationship between insulin IT and hypoglycemia. Third, the study was performed in Nanjing, China and involved Chinese patients only; care should be taken in extrapolating the results to diabetes patient populations in other countries.

CONCLUSIONS

Insulin IT was negatively correlated to short- and long-term glycemic control in patients with T2D receiving premixed insulin.

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Authorship. All the named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Authorship Contributions. JHM, and LY contributed to the conception and design of the study. LY, TJ, BD, YL, RS, XPW, HFD, and XFS conducted the study and collected data. LY contributed to the data analysis. LY, and FFL wrote and gave final approval to the manuscript.

Disclosures. Lu Yuan, Fengfei Li, Ting Jing, Bo Ding, Yong Luo, Rui Sun, Xiuping Wang, Hefeng Diao, Xiaofei Su, Lei Ye, and Jianhua Ma declare that they have no conflict of interest.

Compliance with Ethics Guidelines. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all participants included in the study.

Data Availability. The datasets analyzed for the current analysis are available from the corresponding author on reasonable request.

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